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(54) Title: ELECTRONICALLY-SOLID-PHASE ASSAY BIOMOLECULES

(57) Abstract

Disclosed are materials and methods for detecting biomolecules in samples employing transponders associated with the bead(s) used as the solid phase in the assay, and information pertinent to the assay is encoded on the transponders memory elements. A dedicated read/write device is used remotely to encode or remotely to read the information. The invention can be used in direct or competitive ELISA-type assays, or in multiplex assays for the simultaneous assay of several analytes, including nucleic acids and protein.

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ELECTRONICALLY-SOLID-PHASE ASSAY BIOMOLECULES

BACKGROUND OF THE INVENTION

This invention relates to materials and methods for detecting biomolecules in samples, and more particularly to a particulate solid phase having for encoding information concerning the assay, and to assays employing such a solid phase.

Solid phase assays have been used to determine the presence and/or the concentration of biomolecules, such as proteins, peptides, nucleic acids, including deoxyribonucleic acids (DNA), ribonucleic acids (RNA) and their modified forms, as well as carbohydrates and lipids. Solid-phase assays can be performed in a variety of fluids, e.g., simple buffers, biological fluids, such as blood, serum, plasma, saliva, urine, tissue homogenates, and many others.

In solid phase assays, small beads, or microparticles, are typically used as the solid phase to capture the analyte. Solid phase microparticles can be made of a variety of materials, such as glass, plastic or latex, depending on the particular application. Some solid phase particles are made of ferromagnetic materials to facilitate their separation from complex suspensions or mixtures.

In conventional solid-phase assays, the solid phase mainly aids in separating biomolecules that bind to the solid phase from molecules that do not bind to the solid phase. Separation can be facilitated by gravity, centrifugation, filtration, magnetism, immobilization of molecules onto the surface of the vessel, etc. The separation may be performed either in a single step in the assay or, more often, in multiple steps.

Often, it is desirable to perform two or more different assays on the same sample, in a single vessel

and at about the same time. Such assays are known in the art as multiplex assays. Multiplex assays are performed to determine simultaneously the presence or concentration of more than one molecule in the sample being analyzed, or alternatively, to evaluate several characteristics of a single molecule, such as, the presence of several epitopes on a single protein molecule.

One problem with conventional multiplex assays is that they typically cannot detect more than about five analytes simultaneously, because of difficulties with simultaneous detection and differentiation of more than about five analytes. In other words, the number of different analytes that may be assayed simultaneously is limited by the solid phase.

SUMMARY OF THE INVENTION

This invention overcomes many of these problems by the use of transponders associated with the solid phase beads to index the particles constituting the solid phase. Thus, each individual transponder-containing solid phase particle can be assigned a unique index number, electronically encoded inside the particle, that can be retrieved by the scanner device at any time, e.g., at one time during the assay, at multiple times during the assay, or continuously during the assay. The index number may relate to the time and date on which the assay was performed, the patient's name, a code identifying the type of the assay, catalog numbers of reagents used in the assay, or data describing the progress of the assay, such as temperature during different steps of the assay. The index number may define the nucleotide sequence of the oligonucleotide deposited on the surface of the particle, the catalog number of a DNA fragment deposited on the particle, index numbers of chemical steps which were involved in the chemical synthesis of

an oligonucleotide bound to the particle, or some other relevant characteristics of the deposited molecules.

In an electronically-indexed multiplex assay of this invention, two or more transponders, each encoded with a different index number and constructed to bind a different analyte, are incubated with the sample in a single vessel. After necessary additions, incubations and washes are performed, which are similar to incubations and washes in existing assays, the solid phase is analyzed to detect a label indicative of binding of the analyte to the solid phase, such as fluorescence, color, radioactivity or the like. Solid phase analysis is either preceded or followed by the decoding of the index number on the transponder.

Determination of the label and decoding of the memory of the transponder can be done manually on two different instruments, such as a fluorometer and a dedicated scanner, although a single automated instrument that would perform both functions may be used. Such an instrument can be a modified fluorometer in which the scanner is mounted in the proximity of the fluorometer readout window, and reading the sample fluorescence and decoding the transponder are coordinated by a central computer. In addition, such an instrument can be equipped with an automated transport system for transponders.

In one aspect, the present invention provides an electronically-indexed solid phase particle for use in solid phase assays for biomolecules, including proteins and nucleic acids, comprising a transponder and a member of a biomolecular binding pair attached to the transponder.

In another aspect, the present invention provides a method of detecting biomolecules, including proteins and nucleic acids, in a sample using solid phase particles having transponders.

In another aspect, the present invention includes a kit for detecting biomolecules in a sample

using transponders, comprising assay vessels, a probe reagent, and a labeled conjugate reagent.

In another aspect, the present invention provides kits for detecting nucleic acids in samples, comprising assay vessels, at least one transponder having a nucleic acid probe bound to the transponder, and a labeled reagent to detect binding of sample nucleic acids to the probe.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic representation of a simple assay of this invention, involving proteins.

FIG. 2 is a schematic representation of a simple assay of this invention, involving nucleic acids.

FIG. 3 is a schematic representation of a simple nucleic acid-based assay of this invention utilizing an alternative labeling technique.

FIG. 4 is a schematic representation of a multiplex assay of this invention, involving proteins.

FIG. 5 is a schematic representation of a multiplex nucleic acid-based assay of this invention.

FIG. 6 is a schematic representation of a multiplex nucleic acid-based assay of this invention utilizing an alternative labeling technique.

FIG. 7 is a cross-sectional view of a solid phase particle with a transponder and a primary layer of biomolecules bound to a surface thereof.

FIG. 8 is a diagram of a solid phase particle with a transponder, and a primary layer of a nucleic acid sequence attached to the surface thereof.

FIG. 9 is a schematic diagram of the signal pathway for encoding and decoding data on the transponders.

FIG. 10 is a schematic representation of a miniature transponder.

FIG. 11 is a plan view of a miniature transponder.

FIG. 12 is a plan view of a transport system/analytical instrument for implementing the present invention.

FIG. 13 is a plan view of a modified flow cytometer for high speed analysis of solid phase particles of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

Figure 1 depicts a simple assay of the invention, as implemented for an antigen and an antibody. A solid phase particle 10, with a transponder 12 is derivatized by attaching an antibody 11 to the outer surface 16 of the particle 10. Information concerning the assay, e.g., the assay lot number, is encoded on the transponder, either by the manufacturer of the transponder, or by the user with a remote read/write scanner device (not shown). The derivatized particle 10 is incubated with a sample. Antigen 13 present in the sample is bound by the antibody 11 attached to the particle 10. A second, fluorescent-labeled antibody 15 that binds to the antigen 13 is added to the sample mixture, and the particle 10 is thoroughly washed to remove unbound components. The labeled antibody 15 is detected with a fluorometer to identify those transponders 12 that have antigen 13 bound thereto, and the transponder 12 is decoded using the scanner device (not shown) to retrieve the information encoded thereon.

Figure 2 depicts a simple assay of the invention as implemented for nucleic acids. A solid phase particle 10, with a transponder 12 is derivatized by attaching an oligonucleotide probe 17 to the outer surface 16 of the particle 10. Information concerning the assay, e.g., an index number identifying the patient, is encoded on the transponder, either by the manufacturer of transponder, or by the user with a remote read/write scanner device (not shown). Sample containing target nucleic acid 19 is treated to label

all of the nucleic acid therein. The derivatized particle 10 is placed in a sample, and the sample is heated to cause nucleic acids to dissociate. The sample is then cooled under controlled conditions to cause the nucleic acids to anneal. Target nucleic acids 19 complementary to the oligonucleotide probe 17 anneal to the probe 17. The particle 10 is thoroughly washed to remove unbound components. The labeled target nucleic acid 19 bound to the probe 17 is detected with a fluorometer to identify those transponders 12 that have target nucleic acid 19 bound thereto, and the transponder 12 is decoded using the scanner device (not shown) to retrieve the information encoded thereon.

The detection and decoding steps when assaying for both proteins, as well as nucleic acids, may be done separately or may be done simultaneously. Alternatively, the particles of many samples may be pooled into a vessel in no particular order with mixing allowed, and passed through a reader (not shown) that determines and records the fluorescence and, at the same time, decodes the index number recorded in the transponder 12. It is important to note that when encoding or reading data on a transponder, other transponders must be shielded by a metal barrier or other means to prevent the electromagnetic radiation from reaching such "non-target" transponders.

In an alternative labeling technique, depicted in Fig. 3, a second fluorescent-labeled oligonucleotide probe 15 complementary to a second sequence of the target nucleic acid 13 is added to the sample mixture, to specifically label transponders 12 to which target nucleic acids 13 have bound.

A multiplex assay for protein analytes is depicted in Fig. 4. According to this invention, the assay is conducted in a similar manner to that of Fig. 1 , with two or more transponders 12 in each assay vessel (not shown) to detect more than one analyte

simultaneously. The transponders 12 are divided into two or more classes 12 and 12', each class having a distinct index number identifying the class, and each class having different antibody 11 and 11' bound to the surface 16 of the particle 10 and 10'. Each class of transponder 12, 12' is separately encoded, either by the manufacturer or by the user with a read/write scanner device (not shown), with an index number to identify, e.g., the antibody 11 bound to the surface 16 of the particle 10. Again, it is necessary to shield other, non-target transponders during the encoding process. The transponders 12, 12' are incubated in the sample vessel and antigen 13, 13' binds to the respective antibody 11, 11'. Second fluorescent-labeled antibodies 15, 15' that bind to the antigens 13, 13' are added to the sample vessel to bind to the antigens 13, 13'. The transponders 12, 12' are then washed thoroughly to remove unbound sample components and reagents. The labeled antibody 15, 15' is detected with a fluorometer to identify those transponders 12, 12' that have antigen 13, 13' bound thereto, and the transponders 12, 12' are decoded using the scanner device (not shown) to retrieve the information encoded thereon. The detection and decoding steps may be done separately or may be done simultaneously. Alternatively, the particles 10, 10' may be pooled into a vessel in no particular order with mixing allowed, and passed through a reader (not shown) that determines and records the fluorescence and, at the same time, decodes the index number recorded in the transponder 12, 12'.

A multiplex assay for nucleic acids according to this invention is conducted in a similar manner, as depicted in Fig. 5, with two or more transponders 12 in each assay vessel (not shown) to detect more than one labeled target nucleic acid 19 simultaneously. The transponders 12 are divided into two or more classes 12 and 12', each class having a distinct index number

identifying the class, and each class having a different oligonucleotide probe 17 and 17' bound to the surface 16 of the particle 10 and 10'. Using each class of transponder 12, 12' is separately encoded, either by the manufacturer or by the user with a read/write scanner device (not shown), with an index number to identify, e.g., the sequence of the probe 17 bound to the surface 16 of the particle 10. Again, it is necessary to shield other, non-target transponders during the encoding process. The transponders 12, 12' are added to a sample, and the sample is heated to cause nucleic acids to dissociate. The sample is then cooled under controlled conditions to cause the nucleic acids to re-anneal. Target nucleic acid 19, 19' complementary to the respective probes 17, 17' anneals to the probes 17, 17'. The transponders 12, 12' are then washed thoroughly to remove unbound sample components and reagents. The labeled target nucleic acids 19, 19' are detected with a fluorometer to identify those transponders 12, 12' that have target nucleic acids 19, 19' bound thereto, and the transponder 12, 12' is decoded using the scanner device (not shown) to retrieve the information encoded thereon. The detection and decoding steps may be done separately or may be done simultaneously. Alternatively, the particles 10, 10' may be pooled into a vessel in no particular order with mixing allowed, and passed through a reader (not shown) that determines and records the fluorescence and, at the same time, decodes the index number recorded in the transponder 12, 12'.

In an alternative labeling technique, depicted in Fig. 6, second fluorescent-labeled oligonucleotide probes 15, 15' that bind to second sequences of the target nucleic acids 19, 19' are added to the sample vessel to bind to the target nucleic acids 19, 19'. Alternatively, the label may be a radioisotope, such as 32P,35S, 125I, and the like.

The label may also be a chemiluminescent label, such as a luminol derivative or an acridinium ester, that emits light upon oxidation of a substrate. The label may be an enzyme, such as alkaline phosphatase, catalyzing a reaction employing a precipitating fluorogenic substrate, e.g., attophos (JBL Scientific, San Luis Obispo, CA), a precipitating chromogenic substrate, e.g., 5-bromo-4-chloro-3-indolyl phosphate), or a chemiluminescent substrate, e.g., adamantyl 1,2-dioxetane phosphate (Tropix, New Bedford, MA). Finally, the label may be a bioluminescent enzyme such as luciferin.

The assays of the present invention may be used with a variety of analytes, including covalently modified proteins and peptides, protein or peptide conjugates, small molecules, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), modified nucleic acids and analogs of nucleic acids (in particular protein-nucleic acids, PNAs). The analyte may be a complex of biomolecules, such as a virus particle, a protein-nucleic acid complex, or a protein-hapten complex. The analyte may be a cell, and in such case the relevant molecules that participate in the binding process during the assay are typically cell surface receptors or other elements of the cell wall or membrane. Likewise, the sample may be presented in a variety of forms, such as a solution in a simple buffer, or a complex biological fluid, such as blood, serum, urine, saliva, and many others, or it can be mixed with many other analytes which are simultaneously being assayed for in the multiplex format. The target nucleic acid can be mixed with many other analytes. The purity of the nucleic acid deposited as a primary layer on the surface of the transponder can vary as well, from unpurified, partially purified to pure compounds.

The biomolecules deposited as a primary layer on the surface of the transponder may take a variety of forms, as well, such as covalently modified proteins

and peptides, protein or peptide conjugates, small molecules (haptens), ribonucleic acid (RNA), modified nucleic acids and analogs of nucleic acids (in particular protein-nucleic acids, PNAs). The biomolecules can be made in vivo, or in an enzymatic reaction in vitro, or chemically synthesized, either directly or through combinatorial synthesis, or may be a fragment of any of the above products. A preferred example of a product of an enzymatic reaction in vitro is the nucleic acid obtained from the polymerase chain reaction (PCR). The purity of the biomolecules deposited as a primary layer on the surface of the transponder can vary as well, from unpurified, partially purified to pure compounds. The biomolecules, their complexes and aggregates, including subcellular structures or cells, can be deposited as a primary layer on the surface of the transponder by a variety of means including, for example, chemical conjugation to an active group on the support, direct chemical synthesis, adhesion or non-specific binding through hydrophobic interactions.

Figure 7 depicts a solid phase particle 10 for use in the present inventive methods, as applied to protein antigens. The solid phase particle 10 comprises a glass bead with a transponder 12 associated with it, and a member of a biomolecular binding pair (e.g., an antibody or an antigen) attached to the surface 16 of the particle 10 as a primary layer 14. The glass surface 16 of the beads is derivatized through aminoalkylsilane treatment and addition of a cross-linker, to provide primary amine groups on a solid support for further derivatization. The transponder 12 is equipped with a memory element.

Fig. 8 depicts a solid phase particle 10 of the present invention as applied to nucleic acids, having a transponder 12, and a primary layer 14 of an oligonucleotide probe attached to the outer surface 16 of the particle 10.

A transponder is a radio transmitter-receiver activated for transmission of data by reception of a predetermined signal and may also be referred to as a microtransponder, a radio transponder, a radio tag, etc. The signal comes from a dedicated scanner that also receives and processes the data sent by the transponder in response to the signal. The scanner function can be combined with the write function, i.e., the process of encoding the data on the transponder. Such a combination instrument is referred to as a scanner read/write device. An advantage of the transponder-scanner system is that the two units are not electrically connected by wire, but are coupled inductively, i.e., by the use of electromagnetic radiation, typically in the range from 5-1,000 kHz, but also up to 1 GHz and higher.

Figure 9 is a flow chart illustrating the communication between the transponder 12 and a remote scanner read/write device 18. The transponder 12 is encoded with data sent by electromagnetic waves from a remote scanner read/write device 18, unless the transponder 12 was pre-encoded by the manufacturer. After the assay steps are completed, the beads 10 are analyzed to detect the presence of a label indicative of binding of analyte and the transponders 12 are decoded. The scanner 18 sends a signal to the transponder 12. In response to the signal, the transponder 12 transmits the encoded data to the scanner 18.

Some transponders similar to the type employed in the present invention are available commercially. For example, BioMedic Data Systems Inc. (BMDS, 255 West Spring Valley Ave., Maywood, New Jersey) manufactures a programmable transponder for use in laboratory animal identification. The transponder is implanted in the body of an animal, such as a mouse. The transponder is glass-encapsulated to protect the electronics inside the transponder from the

environment. One of the types of transponders manufactured by this corporation, model IPTT-100, has dimensions of 14 x 2.2 x 2.2 mm and weighs 120 mg. The transponder is user-programmable with up to 16 alphanumeric characters, the 16th letter programmable independently of the other 15 letters. It has a built-in temperature sensor as well. The electronic animal monitoring system (ELAMS) includes also a scanner read/write system, such as the DAS-5001 console system, to encode or read data on/from the transponder. The construction of the transponder and scanner is described in U.S. Patent Nos. 5,250,944, 5,252,962, and 5,262,772, the disclosures of which are incorporated herein by reference. Other similar transponder-scanner systems include multi-memory electronic identification tag (U.S. Patent 5,257,011) by AVID Corporation (Norco, CA) and a system made by TEMIC-Telefunken (Eching, Germany). AVID's transponder has dimensions of 1 mm x 1 mm x 11 mm, and can encode 96 bits of information. The present invention can be practiced with different transponders, which might be of different dimensions and have different electronic memory capacity.

The commercially-available transponders are relatively large in size. The speed at which the transponders may be decoded is limited by the carrier frequency and the method of transmitting the data. In typical signal transmission schemes, the data are encoded by modulating either the amplitude, frequency or phase of the carrier. Depending on the modulation method chosen, compression schemes, transmission environment, noise and other factors, the rate of the signal transmission is within two orders of magnitude of the carrier frequency. For example, a carrier frequency of 1,000 Hz corresponds to rates of 10 to 100,000 bits per second (bps). At the rate of 10,000 bps the transmission of 100 bits will take 0.01 sec. The carrier frequency can be several orders of

magnitude higher than 1,000 Hz, so the transmission rates can be proportionally higher as well.

Therefore, the limiting factor in the screening process is the speed at which the transport mechanism carries the transponders through the read window of the fluorometer/scanner device. In state-of-the-art flow cytometers, the rate of movement of small particles or cells is 104-105 per second. A flow cytometer may be used to practice the present invention, if two conditions are met: (1) the transponders are small enough to pass through the flow chamber, and (2) the design of the flow chamber of the flow cytometer is modified to include an antenna and scanner for collecting the electromagnetic radiation emitted by transponders.

A miniature transponder is depicted in Figs. 10 and 11. The source of the electrical power for the transponder 12a is at least one photovoltaic cell 40 within the transponder 12a, illuminated by light, preferably from a laser (not shown). The same light beam induces the fluorescence of fluorogenic molecules immobilized on the surface of the transponder 12a. The transponder 12a includes a memory element 42 that may be of the EEPROM type. The contents of the memory is converted from the digital form to the analog form by a Digital-to-Analog converter 44 mounted on the transponder 12a. The signal is amplified by an amplifier 45, mixed with the carrier signal produced by an oscillator 48, and conducted to the outside of the transponder 12a by an antenna 50.

The contents of memory of the miniature transponder can be permanently encoded, e.g., as ROM memory, during the manufacturing process of the transponder, different batches of transponders being differently encoded. Preferably, the memory of the transponder is user-programmable, and is encoded by the user just before, during, or just after the biological material is deposited on the surface of the

transponder. A user-programmable transponder 12a must have the "write" feature enabled by the antenna 50, amplifier 44 and the Analog-to-Digital converter 46 manufactured on the transponder 12a, as well as the dedicated scanner/write device 27.

In a preferred embodiment, the signal from the scanner is transmitted by modulating the intensity of the light illuminating the transponder 12a, which also actuates the photovoltaic cell power source 40.

The advantages of the miniature transponder of Figs. 10 and 11 are several-fold. First, the transponder dimensions are reduced relative to a conventional transponder, because most of the volume of a conventional transponder is occupied by the solenoid. The current design will enable the production of cubic transponders on the order of 0.01 to 1.0 mm, as measured along a side of the cube, and preferably 0.05 to 0.2 mm.

Second, a large number of transponders can be manufactured on a single silicon wafer. As depicted schematically in Fig. 11, a silicon wafer 60 is simply cut to yield active transponders 12a. Third, the transponder, according the new design, will not need the glass capsule as an enclosure, further reducing the size of the transponder. Silicone dioxide (SiO2) would constitute a significant portion of the surface of the transponder, and SiO2 has chemical properties like glass that allow derivatization or immobilization of biomolecules. Alternatively, microtransponders may be coated with a variety of materials, including plastic, latex, and the like.

Finally, most importantly, the narrow focus of the beam of the laser light would enable only one transponder to be active at a time during decoding, significantly reducing noise level. Advanced user-programmability is desirable as well and, preferably, various memory registers are addressable independently,

i.e., writing in one register does not erase the contents of other registers.

Figure 12 shows the analytical instrumentation and transport system used in an embodiment of the present invention. A quartz tube 20 is mounted in the readout window 22 of a fluorometer 24. The quartz tube 20 is connected to a metal funnel 26. The length of the quartz tube 20 is similar to the dimensions of the transponder 12. Transponders 12 are fed into the metal funnel 26, and pass from the funnel 26 into the quartz tube 20, where the fluorescence is read by the fluorometer 24 and the transponder 12 is decoded by the scanner 27, and then exit through a metal tube 28 and are conducted to a collection vessel (not shown). The metal funnel 26 and metal tube 28 are made of metal shield transponders 12 outside of the read window 22 by shielding from the electromagnetic signal from the scanner 27. This shielding prevents the scanner signal from reaching more than one transponder 12, causing multiple transponders 12 to be decoded.

Minimal modification of the fluorometer 24 would be needed in the vicinity of the location that the tube occupies at the readout moment to allow for positioning of the transponder reading device. To assure compatibility with existing assays, the glass surrounding the transponder could be coated or replaced with the type of plastic currently used to manufacture beads.

In a preferred design, depicted in Fig. 13, a modified flow cytometer is used with the miniature transponder of the present invention. A metal coil antenna 30 is wrapped around the flow cell 32 of a flow cytometer 29. The transponders 12a pass through the flow cell 32, and are decoded by the scanner device 27. The signal carrying the data sent from the transponders 12 is amplified by an amplifier 34 and processed by the scanning device 27. As the transponders 12a are

decoded, fluorescence from the transponders 12a is detected and analyzed by the flow cytometer 29.

The examples below illustrate various aspects of this invention.

EXAMPLE 1

PREPARATION OF DERIVATIZED GLASS BEADS HAVING TRANSPONDERS

The outside glass surface of transponders (e.g., manufactured by BMDS) is derivatized in the following process.

1. Aminoalkylsilane treatment

First, the transponders are cleaned by washing with xylene, followed by a 70% ethanol rinse and air drying. Then, the transponders are submerged for about 30 seconds in a 2% solution of aminopropyltriethoxysilane (Cat.# A3648, Sigma, St. Louis, MO) in dry acetone. The glass beads are then sequentially rinsed with dry acetone and distilled water, and then air dried. This procedure is described in Pierce catalog (pp. T314-T315 of the 1994 catalog, Pierce, Rockford, IL).

2. Attachment Of A Linker To Aminoalkylsilane-Treated Glass

The aminoalkylsilane-treated transponders are immersed in a 10 mM solution of a homobifunctional NHS-ester cross-linker, BS3, bis(sulfosuccinimidyl)suberate (Pierce Cat.# 21579, described on p. T159 of the 1994 Pierce catalog) in 100 mM phosphate buffer (pH 7.0-7.4) for 5 to 60 minutes at room temperature. The exact incubation time is optimized for each treatment. The transponders are then rinsed with water, submerged in a 10-100 mM protein solution in 100 mM phosphate buffer (pH 7.4-8.0), and incubated at room temperature for 2-3 hours. The transponders are rinsed three times with 100 mM phosphate buffer (pH 7.4-8.0). The unreacted sites on the glass are blocked by incubating in Blocker

BLOTTO in phosphate-buffered saline (PBS, Pierce, Cat.# 37526) for 2 hrs. The transponders are rinsed three times with 100 mM phosphate buffer (pH 7.4-8.0), and stored in this buffer at 4oC.

The described procedure, found in Enzyme Immunodiagnostics, E. Kurstak, Academic Press, New York, 1986, pp. 13-22, works with many proteins. However, since properties of proteins can differ widely, for some proteins alternative immobilization schemes may have to be used.

EXAMPLE 2

SINGLE ASSAY FOR A PROTEIN ANALYTE

The purpose of this assay is to obtain a qualitative indication of the presence of human chorionic gonadotropin (hCG)in the sample, which in this example is a solution of hCG labeled with fluorescein in PBS buffer. Another purpose is to be able to retrieve during the course of the assay the identification of the source of hCG used to prepare the sample.

The surface of the transponder (model IPTT-100, manufactured by BMDS) is derivatized as in Example 1 by aminoalkylsilane treatment, and the BS3 linker is attached to the aminoalkylsilane treated glass. A monoclonal antibody raised against hCG is then conjugated to the linker to form an embodiment of the solid phase particle of the present invention.

A transponder derivatized with the anti-hCG antibody is immersed in 1 ml of PBS in a test tube. A fluorescein-labeled hCG preparation is added to the test tube. The final concentration is between 50 pg/ml and 50 mg/ml. The transponder is incubated at room temperature for 30 minutes. During that time, six alphanumeric characters constituting the sample lot-number identifying the source of hCG is encoded into the memory of the transponder using a dedicated read/write scanner. Additional information such as the

lot number of the antibody preparation used, or the name of the patient who donated the serum containing hCG may also be encoded on the transponder. After a series of extensive washes over a period of 5 minutes, the transponder is placed in 1 ml of fresh PBS buffer. The transponder is then placed in a fluorometer, and the fluorescence intensity (FI) is measured and recorded. The FI readout is normalized with respect to positive control transponders which were exposed to fluoresceinated bovine serum albumin instead of fluoresceinated hCG. The electronic memory of the transponder is decoded using a dedicated scanner to obtain the lot number of the hCG preparation.

The advantage of using the transponder in this example instead of a prior art solid phase particle is that there is no need to maintain an association between the test tube and the solid phase at all times during the assay. Instead, after electronically recording the lot number, the transponder can be separated from the original container without losing track of the lot number of the sample to which it was exposed. The transponder can be mixed with other transponders exposed to analytes having different lot numbers without losing information about the presence of hCG in the analyte.

EXAMPLE 3

ELECTRONICALLY INDEXED SOLID PHASE ASSAY FOR HUMAN IgA1 AND IgG1

A total of eight transponders (e.g., BMDS model IPTT-100) are derivatized according to the procedure of Example 1, with two antibodies, human IgA1 (Cat.# I2636) and IgG1 (Cat.# I4014) obtained from Sigma (St. Louis, MO). Thus, two groups of transponders carrying these two antibodies are obtained. Four transponders of each of the two groups of transponders are encoded with the index numbers A1, A2, A3 and A4, and G1, G2, G3 and G4, respectively, by the read/write

scanner device (BMDS). The letter corresponds to the type of immunoglobulin used to derivatize the transponder, and the digit gives the tube number. Transponders are distributed into assay tubes, each tube containing one transponder of each type. Thus, tube 1 contains transponders encoded A1 and G1, tube 2 - A2 and G2, etc.

The following set of analytes is prepared at a concentration between 50 pg/ml and 50 mg/ml in PBS:

Analyte 1: Mixture of monoclonal antibody to human IgA1 labeled with FITC (Cat.#F6016), and monoclonal antibody to human IgG1 labeled with FITC (Cat.#F5016).

Analyte 2: Monoclonal antibody to human IgA1 labeled with FITC.

Analyte 3: Monoclonal antibody to human IgG1 labeled with FITC.

Analyte 4: No antibody present.

2 mls of analyte 1 is added to tube 1, containing two transponders, one of each group as described above. Similarly, 2 mls of analyte 2, 3 and 4 are added to tubes 2, 3 and 4, respectively, also containing two transponders each. The tubes are kept at room temperature for 30 minutes, after which the transponders are washed three times with 5 ml PBS buffer. The fluorescence of each of the transponders is quantitated by using a FluorImager (Molecular Dynamics), and the encoding of each transponder is determined by using the read/write device (BMDS).

The assay described is direct, since the concentration of FITC-derivatized antibody is measured. Alternatively, the assay can be configured in a competitive format to measure the concentration of underivatized antibodies, such as those present in sera. Moreover, the number of analytes that can be tested in one tube is limited only by the requirement that the total volume of the transponders needed in the

single tube should not be much larger than the sample volume.

EXAMPLE 4

MULTIPLEX ASSAY FOR ANTIBODIES EMPLOYING PEPTIDES IMMOBILIZED ON TRANSPONDERS

Peptides can be immobilized on the surface of the transponders' glass envelope by either chemical synthesis or conjugation. The glass surface of the transponder (e.g., AVID) is first derivatized with aminopropyltriethoxysilane, creating a suitable solid support for chemical peptide synthesis. The amino groups of the alkyl chains attached to the support are appropriate for initiating peptide synthesis by forming the amide bond with the C-terminal residue of the peptide when standard Fmoc or Boc chemistries are used. The resulting peptide can be deprotected according to standard protocols without cleaving the peptide from the support.

Alternatively, peptides previously synthesized or isolated can be attached to the treated glass surface using the cross-linker and protocol of Example 1. The requirement will be the presence of a primary amine group in the peptide (such as N-terminal amine), or a secondary amine group. The assay configuration is identical to Example 1, except that the proteins (i.e. monoclonal antibodies) of Example 1 are replaced with peptides in this Example.

EXAMPLE 5

A DIAGNOSTIC KIT FOR PERFORMING AN ELECTRONICALLY INDEXED MULTIPLEX ASSAY FOR THE HEPATITIS C VIRUS

The kit is used to simultaneously determine the presence of antibodies to four Hepatitis C Virus (HCV) antigens in human serum or plasma, or in mixtures of purified anti-HCV antibodies prepared in the laboratory. The HCV antigens are as follows: (1) core,

(2) NS3, (3) NS4, N-terminal part, (4) NS4, C-terminal part.

The constituents of the HCV reagent kit are as follows:

1. Reagent A, Specimen Diluent, 10 Mm Tris-HCL, pH 7.5.

Preservative: 0.1% sodium azide.

2. Reagent B, Probe. Goat antibody to human IgG (H+L), conjugated to biotin. Minimum concentration; 0.1 pg/ml. Preservative: 0.1% sodium azide.

3. Reagent C, Conjugate. Rabbit antibody to biotin, conjugated to alkaline phosphatase. Minimum concentration: 0.1 pg/ml. Preservative: 0.1% sodium azide.

4. Reagent D, Chromogen. 5-bromo-4-chloro-3 indolyl phosphate (0.1%). Preservative: 0.1% sodium azide.

5. 20 Test Vessels. Each vessel is a 2 ml test tube and contains 4 transponders conjugated to four HCV antigens. The antigens are applied at a minimum of 1 ng per transponder. The transponders are electronically encoded with numbers 1,2,3 and 4, corresponding to antigens (1),(2),(3) and (4) respectively.

6. 1 Vial (0.1 ml) Accessory Positive Control. It is an inactivated human plasma containing antibody to HCV, non-reactive for HBsAg and antibody to HIV-I/HIV-2. Minimum titer: 1:2. Preservative: 0.1% sodium azide.

7. 1 Vial (0.1 ml) Accessory Negative Control. It is human plasma nonreactive by FDA licensed tests for antibody to HCV, and non-reactive for HBsAg and antibody to HIV-1/HIV-2. Preservative: 0.1% sodium azide.

8. Wash buffer. 10 mM Tris-HCl, pH 7.5.

9. Enzyme Reaction Buffer, 100 mM Tris-HCl, pH 8.0.

10. Bar coded calibration data sheet.

In an alternative configuration of the kit, the chromogen, reagent 4 above, is replaced with a fluorogen, item 4a and Reagent 4a, namely:

4a. Reagent 4-a, Fluorogen, precipitating substrate for alkaline phosphatase (0.1%). The substrate is attophos reagent, manufactured by JBL Scientific, San Luis Obispo, CA. Preservative: 0.1% sodium azide.

The procedure for performing the assay on a single sample of unknown composition with regard to HCV antibodies is as follows. Three test vessels, X, Y and Z are placed in a rack. Sample is added to vessel X, Accessory Positive Control added to vessel Y, Accessory Negative Control added to vessel Z. Appropriate amounts are determined for each lot of reagents, but approximate volumes are 10-100 ml sample or controls diluted with the Wash Buffer to the final volume of 2 ml. The sample and buffer are thoroughly mixed, and incubated for 30 minutes at room temperature, after which the transponders in the vessel are washed extensively for 5 minutes with the Wash Buffer. Reagent B is then added, and the vessel is incubated for 30 minutes, after which the transponders are washed. Reagent C is then added, and the vessel is incubated for 30 minutes, after which the transponders are washed. One ml of the enzyme reaction buffer is then added to the vessels, followed by 1 ml of the substrate (item 4 or 4a). The contents of the vessels is mixed thoroughly. The vessels are incubated at room temperature for 2 to 30 minutes, depending on the desired sensitivity of the assay, after which the transponders are rinsed with the Wash Buffer to remove excess substrate and that fraction of the product of the reaction which did not precipitate. The color of the transponders is then determined in a photodiode spectrophotometer configured to measure the reflected light, or the fluorescence of the transponders is measured in a fluorometer, depending on the label used.

Each optical measurement is followed by the decoding of the electronic memory of the transponder and associated with the optical measurement.

EXAMPLE 6

MULTIPLEX DNA-BASED ASSAY ON TRANSPONDERS EMPLOYING DNA SYNTHESIZED ON THE SOLID SUPPORT

The glass outer surface of the transponders is first derivatized by an aminoalkylsilane treatment. The transponders (e.g., IPTT-100, BMDS) are cleaned by washing with xylene, followed by a 70% ethanol rinse and air drying. The transponders are then submerged for about 30 seconds in a 2% solution of aminopropyltriethoxysilane (Cat.# A3648, Sigma, St. Louis, MO) in dry acetone. The transponders are then sequentially rinsed with dry acetone and distilled water, and then air dried. This procedure is described in the Pierce catalog (pp. T314-T315 of the 1994 catalog, Pierce, Rockford, IL).

Nucleic acid probes are then covalently linked to the aminoalkylsilane-treated glass by direct chemical synthesis on the glass support. A thymidine-derivatized support containing a stable nucleoside-urethane linkage is prepared, in which 5'-dimethoxytrityl thymidine is reacted with one equivalent of tolylene-2,6-diisocyanate in the presence of one equivalent of N-ethyldiisopropylamine as a catalyst in pyridine/1,2-dichloroethane to generate the monoisocyanate. The monoisocyanate is not isolated, but is reacted directly with the aminopropyltriethoxysilane-derivatized glass surface of the transponders. The procedure is described in detail in B.S. Sproat and D.M. Brown, A new linkage for solid phase synthesis of oligodeoxyribonucleotides, Nucleic Acids Res. 13, 2979-2987, 1985.

The thymidine-derivatized support containing a stable nucleoside-urethane linkage is used directly for the chemical synthesis of oligodeoxynucleotides by

manual synthesis on sintered funnels using standard phosphoramidite-based DNA synthesis reagents, as described in Caruthers, M.H. et al., Deoxyoligonucleotide Synthesis Via The Phosphoramidite Method, Gene Amplification and Analysis, Vol. III (T.S. Papas et al., Eds., Elsevier/North Holland, Amsterdam). The thymidine-urethane linker is resistant to cleavage with base during deprotection, and the resulting product is the deprotected oligonucleotide attached to the glass surface of the transponder through the urethane-thymidilate linker.

The following oligodeoxynucleotide reagents are prepared. Sequence 1 and sequence 2 do not exhibit self-complementarity, are 15 nt long, and are linked to the transponders through a spacer, which is an oligonucleotide having the (dT)10 sequence. Oligonucleotides C and D are derivatized at the 5'-end with fluorescein. The sequences are as follows:

transponder-oligonucleotide A: 5'-spacer-sequence1

transponder-oligonucleotide B: 5'-spacer-sequence2

oligonucleotide C: 5'-fluorescein-sequence1complement

oligonucleotide D: 5'-fluorescein-sequence2complement

Four assay tubes are prepared and labeled 1, 2, 3 and 4, each assay tube to accommodate two transponders, one transponder carrying oligonucleotide A and the second transponder carrying oligonucleotide B. The transponders are electronically encoded with two alphanumeric characters, namely A1,A2,A3,A4 and B1,B2,B3,B4, where the letter corresponded to the oligonucleotide used to derivatize the transponder, and the digit gave the test tube number into which the given transponder is placed. Thus tube 1 contains transponders A1 and B1; tube 2 - A2 and B2; tube 3, A3 and B3; and tube 4, A4 and B4, all immersed in 50 mM Tris-HCl buffer (pH 7.5). Four analytes, X,Y,Z and W, are prepared, as follows. Analyte X contains

oligonucleotide C and oligonucleotide D; Y contains oligonucleotide C only, Z contained oligonucleotide D only, and analyte W does not contain any oligonucleotides. The analyte solutions are prepared in 50 mM Tris-HCl (pH 7.5). The concentration of each given oligonucleotide in the analytes X, Y and Z is 10 nM to 10 mM. After the four tubes are emptied of buffer, but retain the transponders, 2 mls of X,Y,Z and W analyte are added to tubes 1, 2, 3 and 4, respectively. The tubes are heated to 90oC, and slowly cooled to room temperature. Then the transponders are rinsed three times with the buffer. The fluorescence of each transponder is measured on a FluorImager instrument (Molecular Dynamics).

EXAMPLE 7

MULTIPLEX DNA-BASED ASSAY ON TRANSPONDERS EMPLOYING CONJUGATION OF OLIGONUCLEOTIDES TO SOLID SUPPORT

Precleaned transponders (IPTT-100, BMDS) are immersed in a 1% 3-aminopropyltrimethoxysilane solution (Aldrich Chemical, Milwaukee, WI) in 95% acetone/water for 2 minutes, washed extensively with acetone (10 washes, 5 minutes each) and dried (110oC for 45 minutes). The transponders are then treated for 2 hours with 1,4-phenylene diisothiocyanate (Aldrich) (PDC, 0.2% solution in 10% pyridine/dimethyl formamide). The transponders are washed with methanol and acetone and stored at 4oC in an anhydrous environment. The 5'-amino-modified oligonucleotides to be immobilized on the glass support are dissolved in 100 mM sodium carbonate/bicarbonate buffer (pH 9.0) at a concentration of 2 mM, and a 2 ml aliquot is applied directly to the PDC-derivatized transponders and incubated at 37oC in a closed vessel for 2 hours. The transponders are then washed with NH4OH, three times with water and air dried at room temperature. This derivatization procedure is based on a protocol

described in Guo et al. (Direct Fluorescence Analysis Of Genetic Polymorphism By Hybridization With Oligonucleotide Arrays On Glass Support. Nucleic Acids Res. 22, 5456-5465, 1994).

The following oligodeoxynucleotide reagents are prepared. Sequence1 and sequence2 are 15 nt long, and are linked to the transponders through an oligonucleotide spacer having the (dT)10 sequence. Oligonucleotides C and D are derivatized at the 5'-end with fluorescein. The sequences are as follows:

transponder-oligonucleotide A: 5'-spacer-sequence1

transponder-oligonucleotide B: 5'-spacer-sequence2

oligonucleotide C: 5'-fluorescein-sequence1complement

oligonucleotide D: 5'-fluorescein-sequence2complement

Four assay tubes are prepared and labeled 1, 2, 3 and 4, each tube to accommodate two transponders, one transponder carrying oligonucleotide A and the second transponder carrying oligonucleotide B. The transponders are electronically encoded with two alphanumeric characters, namely A1,A2,A3,A4 and B1,B2,B3,B4, where the letter corresponded to the oligonucleotide used to derivatize the transponder, and the digit gave the test tube number into which the given transponder is placed. Thus tube 1 contains transponders A1 and B1; tube 2 - A2 and B2; tube 3, A3 and B3; and tube 4, A4 and B4, all immersed in 50 mM Tris-HCl buffer (pH 7.5). Four analytes, X,Y,Z and W, are prepared, as follows. Analyte X contains oligonucleotide C and oligonucleotide D; Y contains oligonucleotide C only, Z contained oligonucleotide D only, and analyte W does not contain any oligonucleotides. The buffer is 50 mM Tris-HCl (pH 7.5). The concentration of each given oligonucleotide in the analytes X, Y and Z is 10 mM. After the four tubes are emptied of buffer, but retain the transponders, 2 mls of X,Y,Z and W analyte are added to

tube 1,2,3 and 4, respectively. The tubes are heated to 90oC, and slowly cooled to room temperature. Then the transponders are rinsed three times with the buffer. The fluorescence of each transponder is measured on a Fluorimager (Molecular Dynamics).

EXAMPLE 8
CONJUGATION OF STREPTAVIDIN TO THE GLASS SURFACE OF TRANSPONDERS

The outside glass surface of transponders (IPTT-100, BMDS) is derivatized through the aminoalkylsilane treatment outlined above, and a linker is attached to the aminoalkylsilane-treated glass. A variety of methods can be used, as reviewed in Enzyme Immunodiagnostics, E. Kurstak, Academic Press, New York, 1986, pp. 13-22. This procedure a homobifunctional NHS-ester cross-linker, BS3, bis(sulfosuccinimidyl)suberate (Pierce Cat.# 21579, described on p. T159 of the 1994 Pierce catalog).

The transponders are immersed in the 10 mM solution of BS3 in 100 mM phosphate buffer (pH 7.0-7.4) for 5 to 60 minutes at room temperature, and the transponders are rinsed with water. A 10-100 mM streptavidin solution in 100 mM phosphate buffer (pH 7.4 - 8.0) is prepared. The transponders are submerged in the streptavidin solution and incubated at room temperature for 2-3 hours. The transponders are rinsed three times with 100 mM phosphate buffer (pH 7.4-8.0). The unreacted sites on the glass are blocked by incubating in Blocker BLOTTO in PBS (phosphate-buffered saline) (Pierce, Cat.# 37526) for 2 hrs. The transponders are rinsed three times with 100 mM phosphate buffer (pH 7.4-8.0), and stored in this buffer at 4oC.

EXAMPLE 9

DETECTION OF A POINT MUTATION IN THE N-RAS GENE

Point mutations in the N-ras gene are frequently observed in various hematological and solid tumors. A well-characterized mutation is a G -> C mutation in the first position of the 12th codon of the N-ras gene. The present example provides a method to detect this mutation implementing transponders.

The sequence of the first exon of the N-ras gene is given in Table 1. The glass surface of transponders (IPTT-100, BMDS) used in this example is derivatized with streptavidin using the conjugation method described in Example 3. The following oligodeoxynucleotides are chemically synthesized:

(1) GACTGAGTACAAACTGGTGG, corresponding to residues 3-22 of exon 1;

(2) CTCTATGGTGGGATCATATT-biotin, corresponding to residues 111 91;

(3) AACTGGTGGTGGTTGGAGCA, corresponding to residues 14-33,

Oligonucleotide (2) is biotinylated at the 5' end. These sequences were previously used to perform mini-sequencing using scintillating microplates by Ihalainen et al. (BioTechniques, 16, 938-943, 1994). Cellular DNA from patient samples is purified using the standard Blin and Safford procedure (Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY). PCR amplification of DNA using primers (1) and (2) is done on the Perkin-Elmer Cycler 9600, employing 50 cycles of amplification. Each cycle involved a 1 minute denaturation at 94oC, 1 minute annealing at 55oC and 1 minute chain extension at 72OC in a final volume of 100 ml. The single DNA strand carrying biotin is captured on two transponders conjugated to streptavidin by incubating the product of the PCR reaction with the transponders in a buffer containing 150 mM NaCl, 20 mM sodium phosphate (pH 7.4)

and 0.1% Tween-20 at 37oC with gentle shaking for 90 minutes. The bound PCR product was denatured with 50 mM NaOH for 5 minutes at room temperature. The transponders are then washed extensively 3-5 times with a buffer (40 mM Tris-HCl, pH 8.8, 1 mM EDTA, 50 mM NaCl, 0.1% Tween-20). The patient name, consisting of six alphanumeric characters, is encoded on the two transponders using a dedicated read-write scanner.

The diagnostic chain extension reaction is configured for one transponder as follows. The primer, oligonucleotide (3) is at a final concentration of 0.4 M, 3H dCTP or 3H dGTP (Amersham) at 0.2 mM, and 4 units of Taq polymerase, in a final volume of 1 ml of a buffer containing 50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25oC), 0.1% Triton X-100, 4 mM MgCl. The final volume and the test tube type are adjusted depending on the number of transponders so that the whole surface of the transponders is covered with buffer. The reaction is incubated at 55OC for 10 minutes with gentle shaking.

To determine whether the mutation is present, transponders are used in two DNA chain extension reactions. The first reaction contains 3H dCTP and no other dNTPs, the second one contains 3H dGTP and no other dNTPs. Since the transponders are individually encoded with the patient's name, several transponders can be placed in the vessel where the reaction takes place.

After the reactions are completed, the transponders are washed 3 times as described above, and dried for 60 minutes at room temperature. The transponders are subjected to the electronic decoding, which is followed by counting of the radioactivity associated with the transponders in a scintillation counter, with or without scintillation fluid. Radioactivity associated with the reaction employing 3H dCTP indicates the presence of the mutation in the sample DNA.

Table 1

SEQUENCE OF EXON 1 OF THE N-RAS GENE

```
                                                *
ATGACTGAGTACAAACTGGTGGTGGTTGGAGCAGGTGGTGTTGGGAAAAG 50
TACTGACTCATGTTTGACCACCACCAACCTCGTCCACCACAACCCTTTTC
MetThrGluTyrLysLeuValValValGlyAlaGlyGlyValGlyLysSe

CGCACTGACAATCCAGCTAATCCAGAACCACTTTGTAGATGAATATGATC 100
GCGTGACTGTTAGGTCGATTAGGTCTTGGTGAAACATCTACTTATACTAG
rAlaLeuThrIleGlnLeuIleGlnAsnHisPheValAspGluTyrAspP

CCACCATAGAGgtgaggccc 120
GGTGGTATCTCcactccggg
roThrIleGlu
```

Legend to Table 1:

Bold: Oligonucleotides (1) and (2);

Underlined: oligonucleotide primer (3);

Asterisk - indicates the position of the mutation G->C at codon 12. The sequence is from GenBank 86, entry HNSRAS1.

I Claim:

1. An particle for use in solid phase assays for biomolecules, comprising:

(a) a transponder associated with a solid phase particle;

(b) a member of a biomolecular binding pair attached to a surface of the particle.

2. The particle of claim 1, wherein the surface of the particle is glass, latex or plastic.

3. The particle of claim 1, wherein the biomolecular binding pair is an antigen-antibody pair.

4. The particle of claim 1, wherein the biomolecular binding pair is an nucleic acid - nucleic acid pair.

5. The particle of claim 1, wherein at least one member of the biomolecular binding pair is single-stranded nucleic acid.

6. A particle for use in solid phase assays for nucleic acids, comprising a transponder associated with a solid phase particle and a primary layer of streptavidin conjugated to an outer surface of the particle.

7. The particle of claim 6, wherein a biotinylated nucleic acid probe is bound to the primary layer.

8. A method of detecting a member of a biomolecular bindig pair in a sample, comprising the steps of:

(a) providing a solid phase comprising particles having transponders, the transponders

having memory elements and an index number encoded on the memory elements creating at least one class of transponders, each class having a different index number;

(b) the particles having a first member of a biomolecular binding pair attached to a surface of the solid phase particles;

(c) contacting the solid phase with a sample to cause a second member of the biomolecular binding pair to bind to the first member attached to the solid phase;

(d) analyzing the solid phase to detect the presence of a label indicative of binding of the second member; and

(e) decoding the data encoded on transponders using a scanner device to identify the class of transponders to which analytes are bound.

9. A method of claim 8, wherein:

(a) the first member of the biomolecular binding

pair is a nucleic acid probe, and the second member of the biomolecular binding pair is a nucleic acid target, an both the probe and the target having mutually complementary sequences, and

(b) contacting the solid phase with a sample involves:

(bb) denaturing nucleic acids in the sample mixture; and

(bbb) hybridizing nucleic acids in the sample mixture whereby target nucleic acid sequences hybridize to the probe

10. The method of claim 8, wherein the index number is encoded on the transponder memory element by the transponder manufacturer.

11. The method of claim 8, wherein the index number is encoded on the transponder memory element by the user with a scanner device.

12. The method of claim 8 wherein the label is bound to the target nucleic acid.

13. The method of claim 8 wherein the label is bound to a second oligonucleotide probe, the second probe having a sequence complementary to a second target sequence.

14. The method of claim 8, wherein the label comprises a fluorophore, a chromophore, a radiolabel, a chemiluminescent agent or a bioluminescent agent.

15. The method of claim 8, wherein an outer surface of the transponders is glass, plastic or latex.

16. The method of claim 8 wherein the data comprises physical or chemical characteristics or sequences of the biomolecule or biomolecules deposited on solid phase.

17. The method of claim 9 wherein the data comprises characteristics of the sample.

18. A method of detecting biomolecules in a sample, comprising the steps of:

(a) introducing into the sample at least two populations of solid phase particles, each particle having a transponder and having a member of a biomolecular binding pair attached to its surface, a first population having a different biomolecular binding pair member than a second population and the transponders in the first population being encoded with a different

identification than the transponders of the second population;

(b) analyzing the particles to detect a label indicating the binding of a the sample biomolecule; and

(c) decoding the transponders to determine the population of the transponder.

19. The solid phase of claim 18, wherein the solid phase comprises at least three populations of solid phase particles, each particle having a transponder and having an oligonucleotide probe attached to its surface, each of the three populations having a different oligonucleotide probe sequence and each of the populations being encoded with a different identification than the transponders of the second population.

20. A method of performing a multiplex solid phase assay for biomolecules, comprising the steps of:

(a) providing a particulate solid phase, the particles of the solid phase having transponders, the transponders having memory elements encoded with index number creating two or more classes of transponders, each class having a different index number;

(b) contacting the solid phase with a sample and performing a standard assay procedure to cause two or more different analytes to bind to the solid phase;

(c) washing the solid phase to remove unbound sample components;

(d) analyzing the solid phase to detect a label indicative of the presence of bound analytes; and

(e) decoding the data encoded on the transponders to identify the class of transponder to which an analyte is bound.

21. A method of performing a multiplex solid phase assay for target nucleic acids in a sample, comprising the steps of:

(a) providing a particulate solid phase, the particles of the solid phase having transponders, the transponders having memory elements, and an oligonucleotide probe attached to a surface of the particle, the oligonucleotide probe complementary to a target sequence;

(b) the transponders comprising two or more classes of encoded transponders, each class having a different oligonucleotide bound to the surface of the particle, and each class having a different index number encoded on the transponders memory elements;

(c) contacting the solid phase with a sample to form a sample mixture, the sample mixture containing two more transponders of different classes;

(e) denaturing nucleic acids in the sample mixture;

(f) hybridizing nucleic acids in the sample mixture whereby target nucleic acids hybridize to the nucleic acid probe;

(g) removing unbound sample components from the sample mixture;

(h) analyzing the solid phase to detect a label indicative of the presence of bound analytes; and

(i) decoding the data encoded on the transponders to identify the class of transponder to which an analyte is bound.

22. A method of detecting target nucleic acids in a sample, comprising the steps of:

(a) introducing into the sample at least two populations of solid phase particles, each

particle having a transponder and having an oligonucleotide probe attached to its surface, a first population having an oligonucleotide probe that hybridizes to a different target nucleic acid than a second population and the transponders in the first population being encoded with a different identification than the transponders of the second population;

(b) denaturing the nucleic acids in the sample;

(c) hybridizing the target nucleic acids to the oligonucleotide probes;

(d) analyzing the particles to detect a label indicating that target nucleic acid has bound to the probe; and

(e) decoding the transponder to identify the probe.

23. The solid phase of claim 22, wherein the solid phase comprises at least three populations of solid phase particles, each particle having a transponder and having an oligonucleotide probe attached to its surface, each of the three populations having a different oligonucleotide probe sequence and each of the populations being encoded with a different identification than the transponders of the second population.

24. A kit for detecting the presence of a member of a biomolecular binding pair in a sample, comprising:

(a) at least one assay vessel, containing at least one solid phase particle, a transponder associated with the particle, the transponder having a memory element, and a primary layer of biomolecules bound to a surface of the particle;

(b) at least one probe reagent, comprising a member of a biomolecular binding pair;

(c) at least one labeled conjugate reagent that binds selectively to the probe reagent.

25. The kit of claim 24, further comprising:

(a) at least one positive control, comprising a solution of a member the biomolecular binding pair; and

(b) at least one negative control, comprising a solution free of the biomolecular binding pair member;

26. The kit of claim 25, further comprising:

(a) a sample diluent buffer solution; and

(b) an enzyme reaction buffer solution.

27. The kit of claim 24, wherein the primary layer comprises protein antigens.

28. The kit of claim 24, wherein the primary layer of biomolecules comprise viral antigens.

29. The kit of claim 24, wherein the biomolecular binding pair member to be detected comprises a cell.

30. A kit for detecting the presence of a nucleic acid in a sample, comprising:

(a) at least one assay vessel, containing at least one solid phase particle having a transponder, and an oligonucleotide probe bound to a surface of the particle; and

(b) at least one label reagent.

31. The kit of claim 30, wherein the label reagent comprises a reagent that labels the target nucleic acid.

32. The kit of claim 30, wherein the label reagent comprises a second labeled oligonucleotide probe complementary to a second target sequence.

33. The kit of claim 30, further comprising:

(a) at least one positive control, comprising a solution of solution of nucleic acid complementary to the oligonucleotide probe bound to the particle; and

(b) at least one negative control, comprising a solution free of nucleic acids;

34. The kit of claim 27, further comprising:

(a) a sample diluent buffer solution; and

(b) an enzyme reaction buffer solution.

Fig. 1

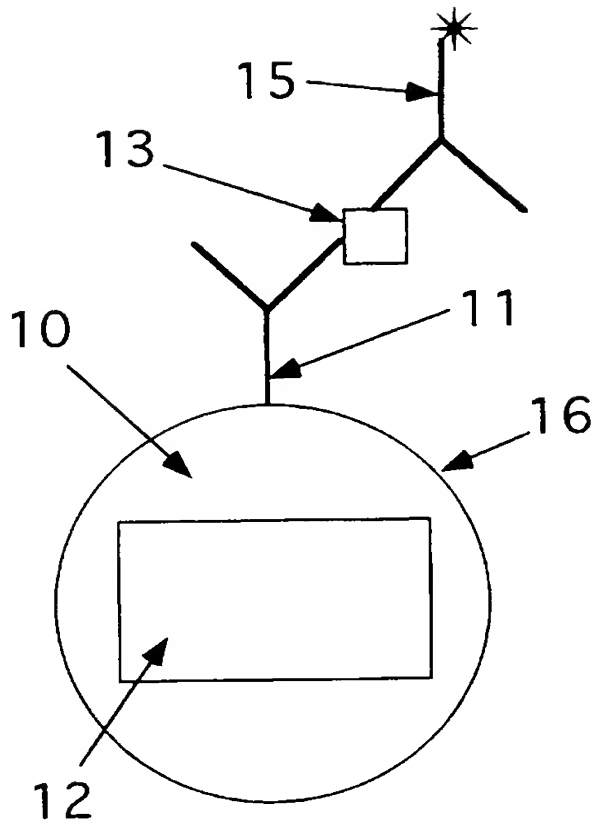


Fig. 2

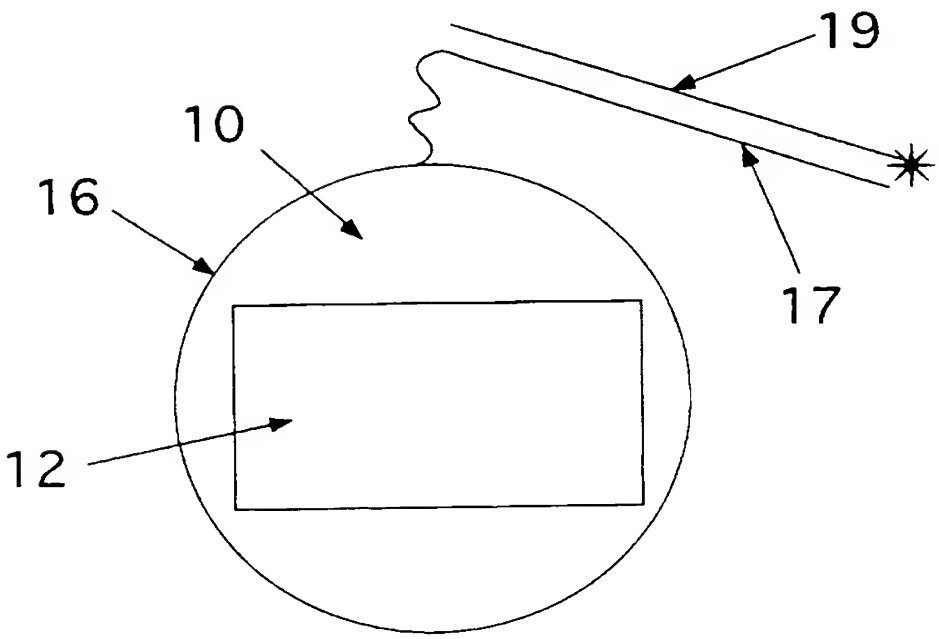


Fig. 3

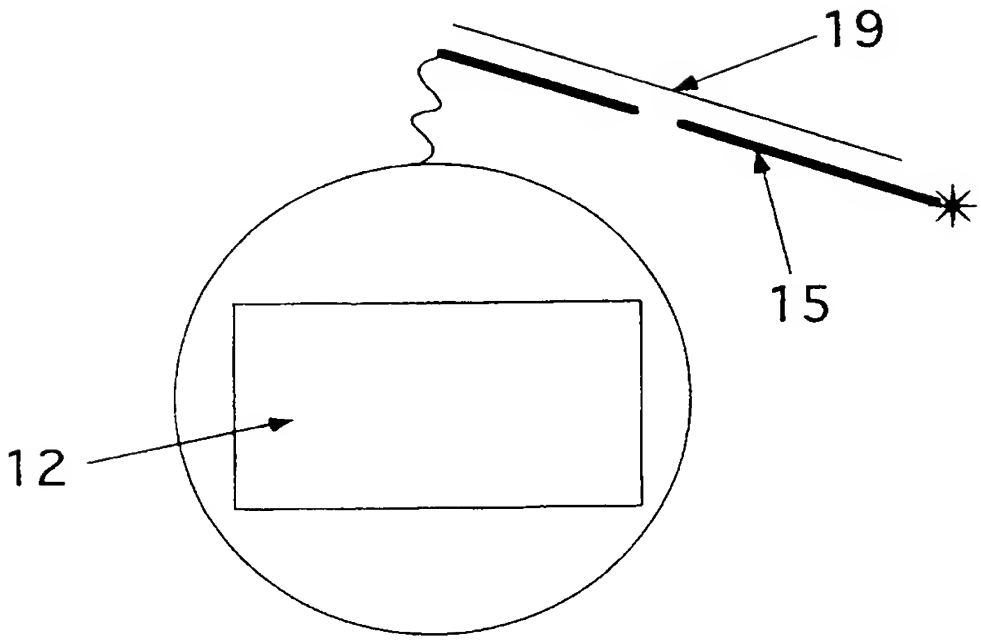


Fig. 4

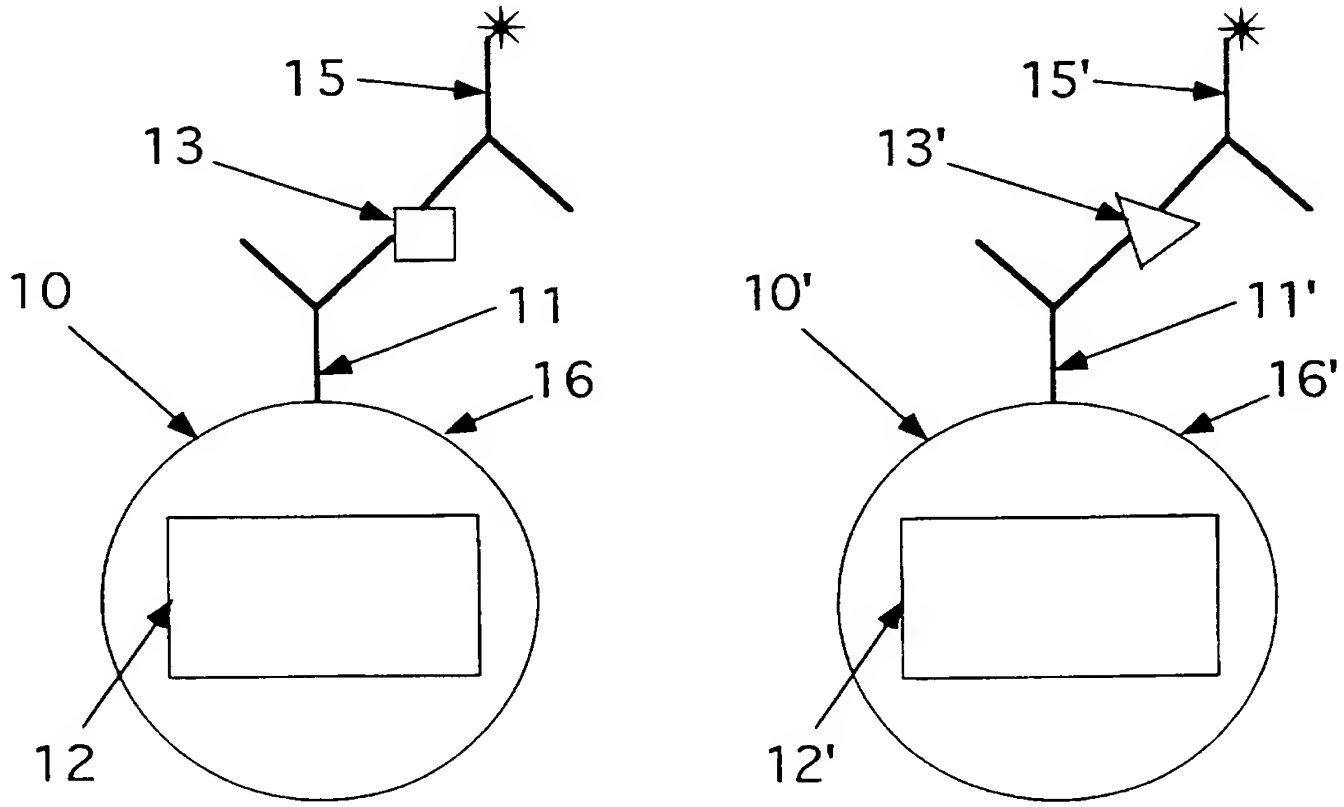


Fig. 5

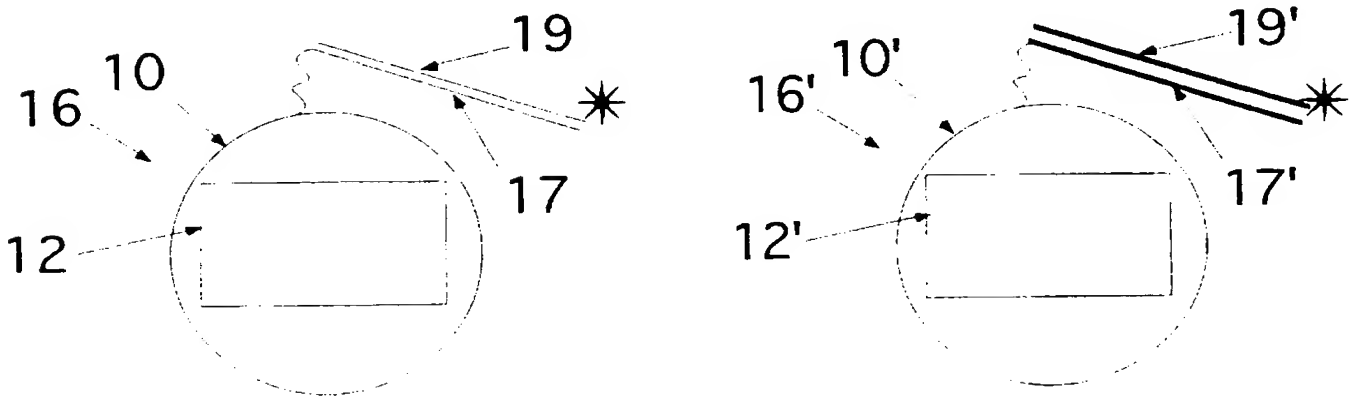


Fig. 6

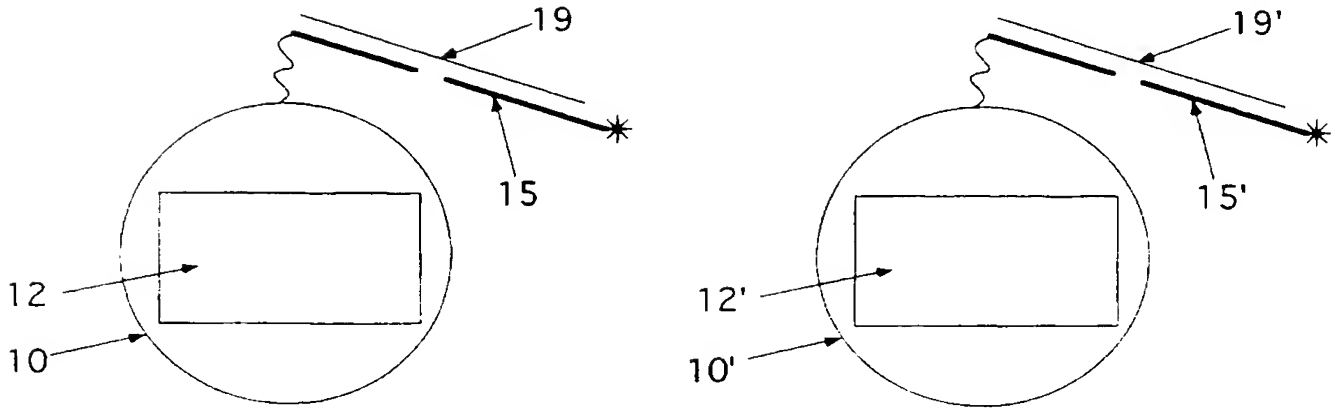


Fig. 7

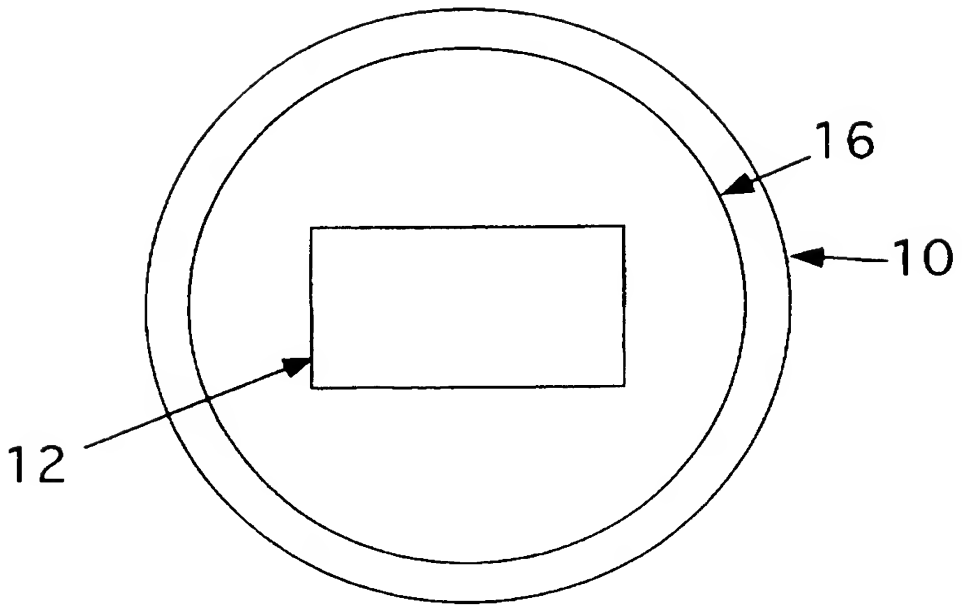


Fig. 8

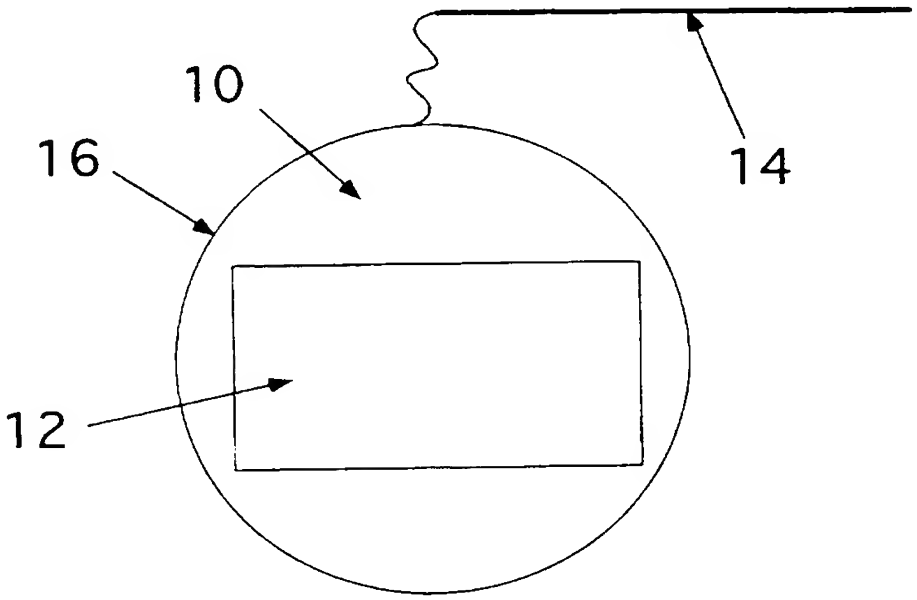


Fig. 9

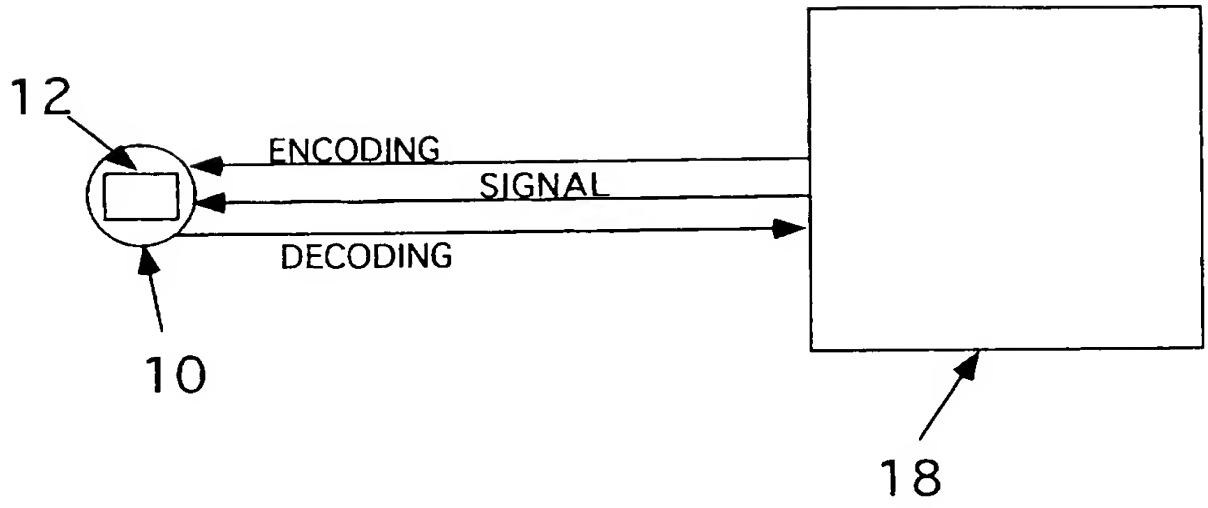


Fig. 10

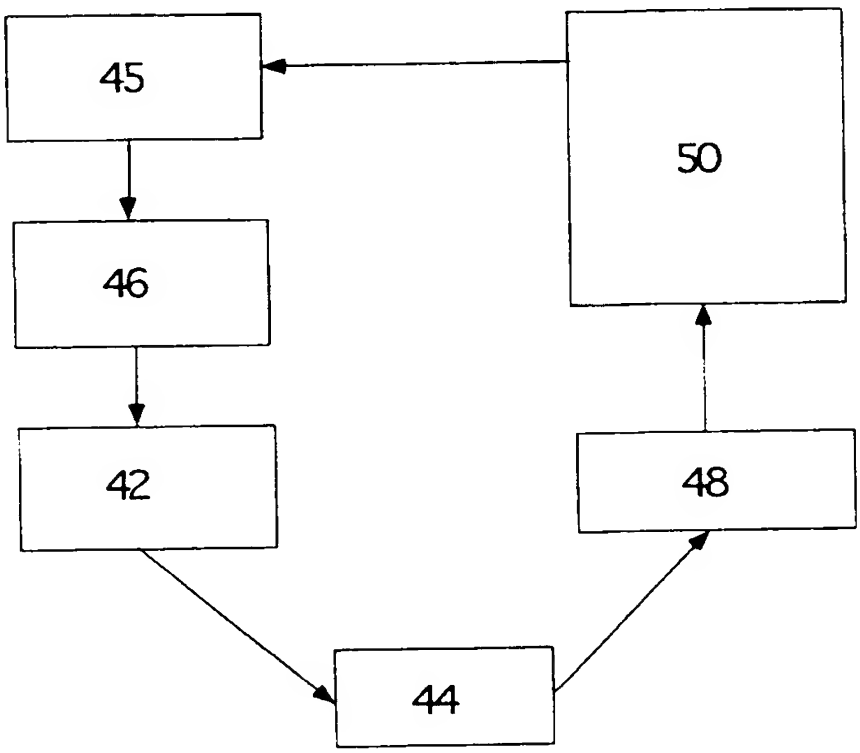


Fig. 11

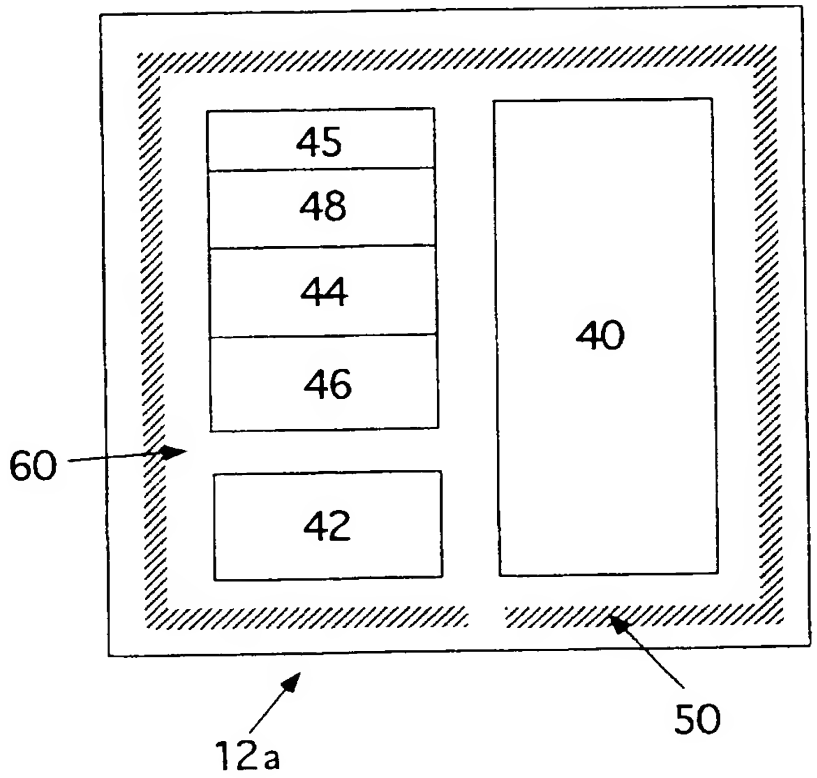


Fig. 12

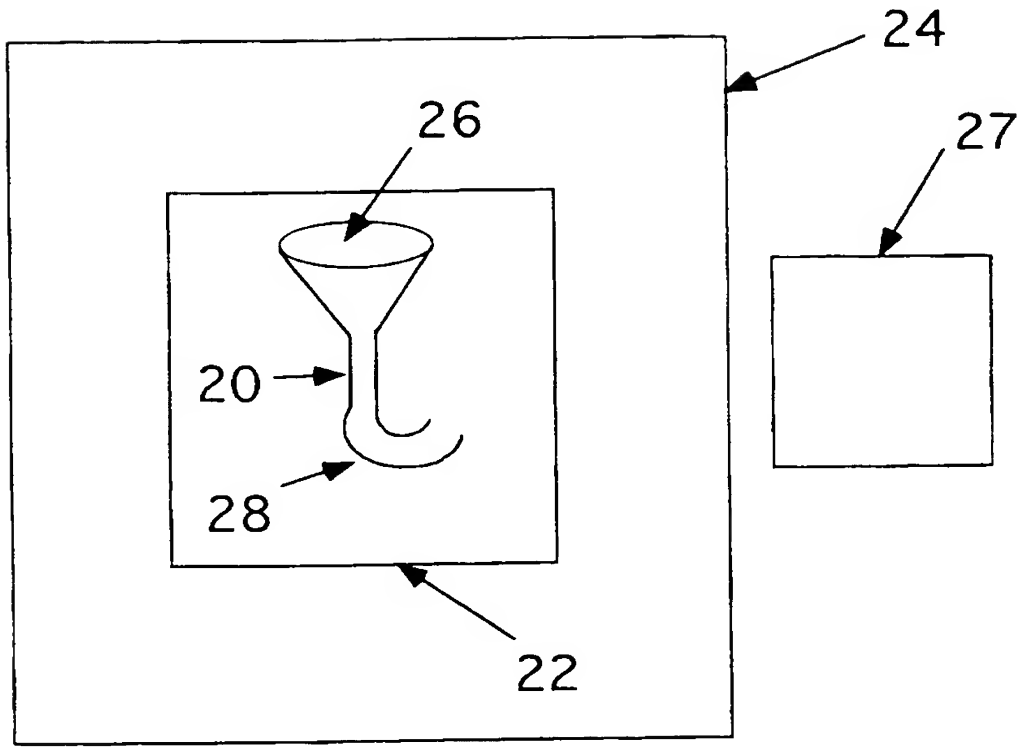
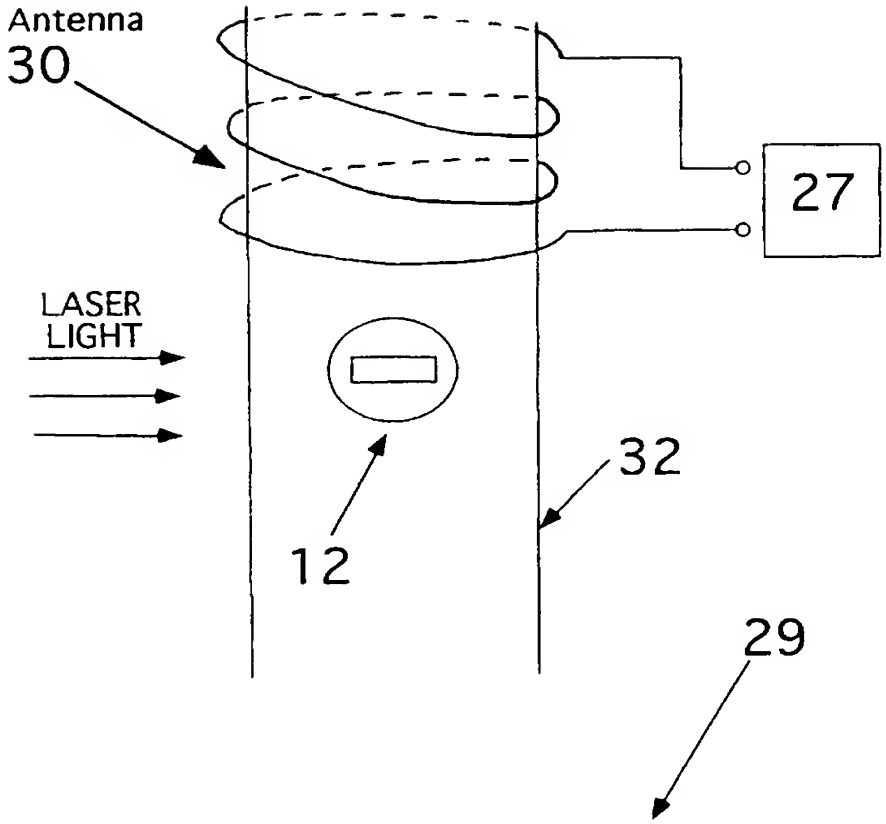


Fig. 13



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/18939

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12Q 1/68; C12P 19/34
US CL :435/6; 435/91.2
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6; 435/91.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,297,337 A (MANSFIELD ET AL.) 27 October 1981, see entire document.	1-34
Y	US 4,177,253 (DAVIES ET AL.) 04 December 1979, see entire document.	1-34
Y	US 4,452,773 A (MOLDAY) 05 June1984, see entire document.	1-34
Y	Urdea et al. A comparison of non-radisotopic hybridization assay methods using fluorescent, chemiluminescent and enzyme labeled synthetic oligodeoxyribonucleotide probes, Nucleic Acid Research. 1988, Volume 16, NO. 11, pages 4937-4956. see entire article.	1-34

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 01 FEBRUARY 1997	Date of mailing of the international search report 07 MAR 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer EGGERTON CAMPBELL Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/18939

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Service radio tags speed compound synthesis. Science 27 October 1995, Volume 270, page 577. see entire document.	1-34
Y	Albretsen et al, Applications of magnetic beads with covalently attached oligonucleotides in hybridization: Isolation and detection of specific measles virus mRNA from a crude cell lysate. Analytical Biochemistry. 1990. Volume 189, pages 40-50, see entire document.	1-34
Y	Maskos et al. Oligonucleotide hybridisations on glass supports: a novel linker for oligonucleotide synthesis and hybridisation properties of oligonucleotides synthesized IN SITU. Nucleic Acid Research. 1992. Volume 20. NO. 7. pages 1679-1684, see entire document.	1-34